REMARKS/ARGUMENTS

Claims 38, 40-42, 62 and 75 are pending. Claims 38, 40 and 62 have been amended herein. Claims 1-37, 39 43-61 and 63-74 have been cancelled without intending to abandon or to dedicate to the public any patentable subject matter. As set forth more fully below, reconsideration and withdrawal of the Examiner's rejections of the claims are respectfully requested.

Objection to the Specification

The Examiner has objected to the specification as containing trademarks that are not capitalized. The Specification has been amended herein to overcome this objection.

The Examiner has also objected to the title of the invention. Applicants will change the title of the invention after the identification of any allowable subject matter.

Rejections Under 35 U.S.C. § 112, First Paragraph

A. Claims 38, 40-42 and 62, as amended, are enabled by the specification.

The Examiner has rejected Claims 38-42 and 62 under 35 U.S.C. § 112, first paragraph, as lacking enablement for all protein variants, cytokine variants, and 4-alpha helix bundle cytokines comprising the substitution of valine for phenylalanine.

Specifically, the Examiner argues that, while the specification is enabling for TPO muteins and EPO muteins, the assertion that all phenylalanine to valine substitution muteins of all protein variants, cytokine variants and 4-alpha helix bundle cytokines will have biological activities similar to the TPO muteins and EPO muteins cannot be accepted in the absence of direct supporting evidence, which is not provided in the specification, because the relevant literature reports examples of polypeptide families wherein individual members have distinct and sometimes even opposite biological activities. Applicants respectfully traverse this rejectin based on the asserted lack of enablement for the following reasons.

The binding affinity between a cytokine and the corresponding receptor depends upon the physical properties of the amino acids themselves, including their hydrophobic index and size. In accordance with the present invention, a cytokine variant which substitutes valine for phenylalanine residues in a binding domain will have enhanced physiological activity modulating effects for reasons related to the physical properties imparted by the amino acid substitution as follows:

1) Phenylalanine is a relatively nonpolar amino acid that has an aromatic side chain and a hydrophobicity index of 3.0. Valine is a nonpolar, hydrophobic amino acid that has an aliphatic side chain and a hydrophobicity index of 4.0. Additionally, because valine is smaller than phenylalanine, a cytokine that has substitutions of valine for phenylalanine residues will fit deeper in the binding pocket of the corresponding receptor. Thus, a cytokine that substitutes valine for phenylalanine residues in the binding domain will have an increased hydrophobic force and will be positioned deeper in the receptor binding site, leading to an increased binding affinity, in turn leading to an increased biological response modulating efficiency.

2) The binding domains of proteins generally have a hydrophilic region at the NH-ward and COOH-ward sites of the hydrophobic binding region. In all cases, protein binding in the hydrophilic region occurs first, followed by binding in the inner, hydrophobic region. This process is true of all proteins, including cytokines. The substitution of valine for phenylalanine in the hydrophobic region readily produces a variant having a high binding affinity. Thus, the substitution of valine for phenylalanine within the hydrophobic core of binding domain changes cytokines, including 4-alpha helix bundle cytokines, into nonpathogenic mutants having higher binding affinities.

3) The substitution of valine for phenylalanine residues, as a conservative substitution, has minimal influence on the secondary or tertiary structure of a protein and thus rarely affects the function of the protein. Further, because phenylalanine is mainly present in a highly hydrophobic region, it is rarely exposed to the exterior of the protein in typical physiological fluids. When phenylalanine residues are replaced by valine, the modified protein becomes

smaller and more tightly compressed in tertiary structure due to the higher hydrophobicity of valine. For this reason, the valine-for-phenylalanine modified protein exhibits less potential to illicit an antibody reaction.

For these reasons, the enhanced receptor binding effects described in the present invention are achieved with any cytokine, including 4-alpha helix bundle cytokines, and the modified cytokines exhibit higher binding affinity and biological activity than wild type cytokines, while avoiding the problems associated with conventional protein mutants, such as antibody production.

Additional direct evidence that a substitution of valine for phenylalanine residues leads to increased binding affinity is supported by the finding of FcγRIIIa (CD16) mutation expressed on NK cells in human autoimmune diseases as described by Jianming Wu et al. (Clinical Investigations, 100:1059-70 (1997), a copy of which is enclosed here for the Examiner's convenience). As described in this research, the human receptor protein has a genetic polymorphism such that two groups of individuals exist: one group has phenylalanine at position 176 of the receptor (a position recognized as participating in Fc recognition of an antibody ligand), while the other group has valine at this position. Individuals having phenylalanine at position 176 of the receptor exhibit weakened binding affinity for the FC region of the antibody ligand and are highly susceptible to systemic lupus erythematosus (SLE).

Further evidence is found in Tim Clackson et al. (PNAS USA 95:10437 (1998) a copy of which is enclosed with this response for the Examiner's convenience). This reference describes the affinity of intracellular proteins with a FKBP sequence in the binding domain for certain organic compounds. The authors show that the binding affinity of a modified FKBP protein having a truncated phenylalanine residue in the binding domain for a modified organic compound increases thousands of times over the affinity of the wild type protein for the wild type ligand. These data show that any proteins, including 4-alpha helix bundle cytokines, can have substantially increased binding affinity following minor changes to phenylalanine residues within the binding site.

A. Claims 38, 40-42 and 62, as amended, do not require undue experimentation for one of skill in the art.

The Examiner has also rejected Claims 38-42 and 62 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Examiner notes that the specific examples of nine different TPO and EPO muteins provided in the specification sufficiently describes the use of TPO and EPO cytokines in the methods of the present invention but argues that the direction or guidance presented in the specification is insufficient to support the production and use of all cytokine variants having a valine for phenylalanine substitution, and that one of skill in the art would be required to undertake undue experimentation to modify and test all cytokines of interest. It is the Examiner's position that while the required methods are well known and the skills of those of the relevant art are high, the skilled artisan could only carry out the claimed methods with the two proteins for which specific examples are provided.

Applicants traverse this rejection based on the Examiner's asserted lack of experimental evidence. Applicants submit that, contrary to the Examiner's assertion, undue experimentation is not viewed solely in light of the examples provided in the specification. Indeed, as stated in the guidance provided by section 2164.02 of the MPEP:

> The presence of only one working example should never be the sole reason for rejecting claims as being broader than the enabling disclosure, even though it is a factor to be considered along with all the other factors. To make a valid rejection, one must evaluate all the facts and evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims.

In this instance, the Examiner is arguing that the reason the highly skilled artisan could not use the working examples provided to extrapolate to the entire scope of the claims is because the examples show differing activity within the TPO and EPO muteins produced and because there is a general art acknowledgment that knowledge of protein structure does not, by itself,

predict protein function. Thus, the Examiner argues, it would take too much experimentation by the skilled artisan to make any modified protein and determine whether it is functional and, further that the specification does not teach the skilled artisan that Applicants had successfully practiced the invention and described the same at the time of filing the instant application.

But the *work required* to produce these mutants is distinct from *requiring too much experimentation*. The methodology used to arrive at the modified cytokines is well known, and the relevant skill in the art is high, and, as Applicants have described above, the outcome of enhanced receptor binding affinity is known and expected. Thus, there is little experimentation necessary for one of skill in the art to carry out the known methods necessary to practice the presently claimed invention. However, Applicants agree with the Examiner that these known methods are labor intensive and require the skilled artisan to perform laboratory work to practice the invention. But this labor, and the work necessary, are not experimentation with an unknown outcome - instead, they are merely the application of known methods by skilled artisans in which the outcome has been shown by working examples in the instant specification. Thus, the Examiner's review of the 'Wands' factors' confuses a labor intensive method with undue experimentation to conclude that the currently claimed methods are not enabled by the present specification. Applicants submit that too much work is not to be equated with undue experimentation and that the working examples and accompanying description provide adequate enablement for the currently claimed methods.

Rejections Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected Claims and under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, the Examiner argues that the recitation of "positions 110 and 180" and "of an amino acid sequence designated as SEQ ID NO.: 25" in Claims 42 and 62, respectively, are indefinite as they are unclear as to the actual intended amino acid residues amongst the 4-helix bundle cytokines and their derivatives and the specific

sequence of reference. Applicants have amended both claims to specify that the amino acid residue positions recited in these claims are made with reference to the specific binding domain made in SEQ ID NO.: 25 or with reference directly to SEQ ID NO.: 25. Applicants therefore submit that the recitation of the starting materials is sufficiently definite to meet the requirements of 35 U.S.C. § 112, second paragraph.

Claim Rejections Under 35 U.S.C. § 102

The Examiner has rejected Claim 38 under 35 U.S.C. § 102(b) as being anticipated by Smulevich et al., (Biochemistry 33(23):7398-7407 (1994)). Applicants have amended Claim 38 to restrict the claimed protein variant to a protein having a cytokine binding domain containing a valine-for-phenylalanine substitution within the binding domain. For this reason, Applicants submit that Smulevich et al. does not anticipate Claim 38, as amended. Applicants therefore respectfully request the Examiner's rejection under 35 U.S.C. § 102(b) be withdrawn.

Based upon the foregoing, Applicants believe that all pending claims are in condition for allowance and such disposition is respectfully requested. In the event that a telephone conversation would further prosecution and/or expedite allowance, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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